

# Examiner's reference ①

4 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 1994:531023 BIOSIS  
DN PREV199497544023  
TI Strain selection, taxonomy, and genetics of xylose-fermenting yeasts.  
AU Jeffries, T. W. (1); Kurtzman, C. P.  
CS (1) Forest Prod. Lab., U.S. Dep. Agric., Forest Serv., Madison, WI 53705  
USA  
SO Enzyme and Microbial Technology, (1994) Vol. 16, No. 11, pp. 922-932.  
ISSN: 0141-0229.  
DT General Review  
LA English  
AB Xylose utilization is essential for the efficient conversion of lignocellulose to ethanol. The objective of this review is to trace the development of **xylose-fermenting yeast** strains from their discovery in 1980. Following initial reports, screens of known yeasts identified five species of interest: *Candida shehatae*, *Candida tenuis*, *Pachysolen tannophilus*, *Pichia segobiensis*, and *Pichia stipitis*. *Candida shehatae* strains can be divided into three varieties. *Pachysolen tannophilus* and *Pichia stipitis* have been studied most extensively and have the best-understood genetic systems. Improved mutants of *P. tannophilus* have been obtained by selecting for an inability to oxidize ethanol (eth) and for rapid growth on xylitol and nitrate. Improved *P. stipitis* mutants have been obtained by selecting for flocculation, decreased utilization of glucose, and growth on noninductive carbon sources. Bacterial xylose isomerase has been cloned and expressed in *S. cerevisiae* and *Schizosaccharomyces pombe*, but the heterologous enzyme is inactive. Xylose reductase and xylitol dehydrogenase have been cloned from *P. stipitis* and expressed in *Saccharomyces cerevisiae*, giving rise to transformant *S. cerevisiae* that grow on xylose but that ferment it poorly. A transformation and expression system based on the URA3 marker has recently been developed for *P. stipitis* so that contemporary genetic methods may be brought to bear on this organism.  
CC General Biology - Taxonomy, Nomenclature and Terminology \*00504  
General Biology - Conservation, Resource Management \*00512  
Cytology and Cytochemistry - Plant \*02504  
Genetics and Cytogenetics - Plant \*03504  
Comparative Biochemistry, General \*10010  
Biochemical Methods - General \*10050  
Biochemical Methods - Carbohydrates \*10058  
Biochemical Studies - General \*10060  
Biochemical Studies - Proteins, Peptides and Amino Acids \*10064  
Biochemical Studies - Carbohydrates \*10068  
Biophysics - Molecular Properties and Macromolecules \*10506  
Enzymes - General and Comparative Studies; Coenzymes \*10802  
Enzymes - Methods \*10804  
Enzymes - Chemical and Physical \*10806  
Enzymes - Physiological Studies \*10808  
Metabolism - General Metabolism; Metabolic Pathways \*13002  
Metabolism - Energy and Respiratory Metabolism \*13003  
Metabolism - Carbohydrates \*13004  
Nutrition - Carbohydrates \*13220  
Food and Industrial Microbiology - Biosynthesis, Bioassay and Fermentation  
\*39007  
Botany, General and Systematic - Fungi \*50506

24 8/4

Plant Physiology, Biochemistry and Biophysics - Nutrition \*51504  
Plant Physiology, Biochemistry and Biophysics - Respiration, Fermentation \*51508  
Plant Physiology, Biochemistry and Biophysics - Growth, Differentiation \*51510  
Plant Physiology, Biochemistry and Biophysics - Enzymes \*51518  
Plant Physiology, Biochemistry and Biophysics - Metabolism \*51519  
BC Fungi - Unspecified \*15000  
IT Major Concepts  
    Biochemistry and Molecular Biophysics; Bioenergetics (Biochemistry and Molecular Biophysics); Bioprocess Engineering; Cell Biology; Conservation; Development; Enzymology (Biochemistry and Molecular Biophysics); General Life Studies; Genetics; Metabolism; Methods and Techniques; Nutrition; Systematics and Taxonomy  
IT Chemicals & Biochemicals  
    ETHANOL; ALCOHOL; XYLOSE; CELLULOSE  
IT Industry  
    biotechnology industry  
IT Miscellaneous Descriptors  
    ALCOHOL PRODUCTION; CELLULOSE CONVERSION; ENZYMES; ETHANOL PRODUCTION; FERMENTATION; GENETIC METHODS; GROWTH; NUTRITION; XYLOSE UTILIZATION  
ORGN Super Taxa  
    Fungi - Unspecified: Fungi, Plantae  
ORGN Organism Name  
    fungi (Fungi - Unspecified); fungus (Fungi - Unspecified)  
ORGN Organism Superterms  
    fungi; microorganisms; nonvascular plants; plants  
RN 64-17-5 (ETHANOL)  
64-17-5. (ALCOHOL)  
58-86-6Q (XYLOSE)  
25990-60-7Q (XYLOSE)  
9004-34-6 (CELLULOSE)

Examiner's ref.

L9 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2001 ACS  
AN 1983:124199 CAPLUS  
DN 98:124199  
TI Direct fermentation of D-xylose to ethanol by a **xylose-fermenting yeast** mutant  
IN Gong, Cheng Shung  
PA Purdue Research Foundation, USA  
SO Eur. Pat. Appl., 21 pp.  
CODEN: EPXXDW  
DT Patent  
LA English  
IC C12P007-06  
CC 16-5 (Fermentation and Bioindustrial Chemistry)  
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 66396	A1	19821208	EP 1982-302474	19820514
	EP 66396	B1	19850821		
	R: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	US 4368268	A	19830111	US 1981-263925	19810515
	US 4511656	A	19850416	US 1982-376731	19820511
	WO 8204068	A1	19821125	WO 1982-US642	19820513
	W: AU, BR, DK, FI, JP, NO				
	AU 8285859	A1	19821207	AU 1982-85859	19820513
	ZA 8203350	A	19830629	ZA 1982-3350	19820514
	AT 15073	E	19850915	AT 1982-302474	19820514
	CA 1207257	A1	19860708	CA 1982-402984	19820514
PRAI	US 1981-263925		19810515		
	US 1982-376731		19820511		
	WO 1982-US642		19820513		
	EP 1982-302474		19820514		
AB	EtOH [64-17-5] is produced from D-xylose [58-86-6] or hemicellulose hydrolyzate by <i>Candida</i> or <i>Saccharomyces cerevisiae</i> mutants. Thus, <i>S. cerevisiae</i> ATCC 20618 was inoculated into pH 5.6 YM medium contg. 5% xylose and incubated at 30.degree. for 48 h with shaking.				
	The concn. of EtOH was 1.41%.				
ST	<i>Saccharomyces</i> ethanol fermn xylose hemicellulose; <i>Candida</i> ethanol fermn xylose hemicellulose; yeast ethanol fermn xylose hemicellulose				
IT	<i>Candida</i> (ethanol manuf. from hemicellulose hydrolyzate and xylose with)				
IT	<i>Saccharomyces cerevisiae</i> (ethanol manuf. from xylose with)				
IT	Fermentation (ethanol, of hemicellulose hydrolyzate and xylose with yeast)				
IT	58-86-6, biological studies 9034-32-6D, hydrolyzates				
RL	BIOL (Biological study)				
	(ethanol manuf. from, by yeast)				
IT	64-17-5P, preparation				
RL	BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)				
	(manuf. of, from xylose by yeast)				

L6 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 1994:531023 BIOSIS  
DN PREV199497544023  
TI Strain selection, taxonomy, and genetics of xylose-fermenting yeasts.  
AU Jeffries, T. W. (1); Kurtzman, C. P.  
CS (1) Forest Prod. Lab., U.S. Dep. Agric., Forest Serv., Madison, WI 53705  
USA  
SO Enzyme and Microbial Technology, (1994) Vol. 16, No. 11, pp. 922-932.  
ISSN: 0141-0229.  
DT General Review  
LA English  
AB Xylose utilization is essential for the efficient conversion of lignocellulose to ethanol. The objective of this review is to trace the development of **xylose-fermenting** yeast strains from their discovery in 1980. Following initial reports, screens of known yeasts identified five species of interest: *Candida shehatae*, *Candida tenuis*, *Pachysolen tannophilus*, *Pichia segobiensis*, and *Pichia stipitis*. *Candida shehatae* strains can be divided into three varieties. *Pachysolen tannophilus* and *Pichia stipitis* have been studied most extensively and have the best-understood genetic systems. Improved mutants of *P. tannophilus* have been obtained by selecting for an inability to oxidize ethanol (eth) and for rapid growth on xylitol and nitrate. Improved *P. stipitis* mutants have been obtained by selecting for flocculation, decreased utilization of glucose, and growth on noninductive carbon sources. Bacterial xylose isomerase has been cloned and expressed in *S. cerevisiae* and ***Schizosaccharomyces pombe***, but the heterologous enzyme is inactive. Xylose reductase and xylitol dehydrogenase have been cloned from *P. stipitis* and expressed in *Saccharomyces cerevisiae*, giving rise to transformant *S. cerevisiae* that grow on xylose but that ferment it poorly. A transformation and expression system based on the URA3 marker has recently been developed for *P. stipitis* so that contemporary genetic methods may be brought to bear on this organism.  
CC General Biology - Taxonomy, Nomenclature and Terminology \*00504  
General Biology - Conservation, Resource Management \*00512  
Cytology and Cytochemistry - Plant \*02504  
Genetics and Cytogenetics - Plant \*03504  
Comparative Biochemistry, General \*10010  
Biochemical Methods - General \*10050  
Biochemical Methods - Carbohydrates \*10058  
Biochemical Studies - General \*10060  
Biochemical Studies - Proteins, Peptides and Amino Acids \*10064  
Biochemical Studies - Carbohydrates \*10068  
Biophysics - Molecular Properties and Macromolecules \*10506  
Enzymes - General and Comparative Studies; Coenzymes \*10802  
Enzymes - Methods \*10804  
Enzymes - Chemical and Physical \*10806  
Enzymes - Physiological Studies \*10808  
Metabolism - General Metabolism; Metabolic Pathways \*13002  
Metabolism - Energy and Respiratory Metabolism \*13003  
Metabolism - Carbohydrates \*13004  
Nutrition - Carbohydrates \*13220  
Food and Industrial Microbiology - Biosynthesis, Bioassay and Fermentation  
\*39007  
Botany, General and Systematic - Fungi \*50506  
Plant Physiology, Biochemistry and Biophysics - Nutrition \*51504  
Plant Physiology, Biochemistry and Biophysics - Respiration, Fermentation \*51508

Plant Physiology, Biochemistry and Biophysics - Growth, Differentiation \*51510  
Plant Physiology, Biochemistry and Biophysics - Enzymes \*51518  
Plant Physiology, Biochemistry and Biophysics - Metabolism \*51519  
BC Fungi - Unspecified \*15000  
IT Major Concepts  
    Biochemistry and Molecular Biophysics; Bioenergetics (Biochemistry and Molecular Biophysics); Bioprocess Engineering; Cell Biology; Conservation; Development; Enzymology (Biochemistry and Molecular Biophysics); General Life Studies; Genetics; Metabolism; Methods and Techniques; Nutrition; Systematics and Taxonomy  
IT Chemicals & Biochemicals  
    ETHANOL; ALCOHOL; XYLOSE; CELLULOSE  
IT Industry  
    biotechnology industry  
IT Miscellaneous Descriptors  
    ALCOHOL PRODUCTION; CELLULOSE CONVERSION; ENZYMES; ETHANOL PRODUCTION; FERMENTATION; GENETIC METHODS; GROWTH; NUTRITION; XYLOSE UTILIZATION  
ORGN Super Taxa  
    Fungi - Unspecified: Fungi, Plantae  
ORGN Organism Name  
    fungi (Fungi - Unspecified); fungus (Fungi - Unspecified)  
ORGN Organism Superterms  
    fungi; microorganisms; nonvascular plants; plants  
RN 64-17-5 (ETHANOL)  
64-17-5 (ALCOHOL)  
58-86-6Q (XYLOSE)  
25990-60-7Q (XYLOSE)  
900

L4 ANSWER 1 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 1996:75338 BIOSIS  
DN PREV199698647473.  
TI Xylulose fermentation by *Saccharomyces cerevisiae* and **xylose-fermenting yeast** strains.  
AU Yu, S.; Jeppsson, H.; Hahn-Hagerdal, B. (1)  
CS (1) Dep. Applied Microbiology, Chemical Centre, Lund Inst. Technol., Univ.  
Lund, P.O. Box 124, S-22100 Lund Sweden  
SO Applied Microbiology and Biotechnology, (1995) Vol. 44, No. 3-4, pp. 314-320.  
ISSN: 0175-7598.  
DT Article  
LA English  
AB Xylulose fermentation by four strains of *Saccharomyces cerevisiae* and two strains of xylose-fermenting yeasts, *Pichia stipitis* CBS 6054 and *Candida shehatae* NJ 23, was compared using a mineral medium at a cell concentration of 10 g (dry weight)/l. When xylulose was the sole carbon source and fermentation was anaerobic, *S. cerevisiae* ATCC 24860 and CBS 8066 showed a substrate consumption rate of 0.035 g g cells-1 h-1 compared with 0.833 gg cells-1h-1 for glucose. Bakers' yeast and *S. cerevisiae* isolate 3 consumed xylulose at a much lower rate although they fermented glucose as rapidly as the ATCC and the CBS strains. While *P. stipitis* CBS 6054 consumed both xylulose and glucose very slowly under anaerobic conditions, *C. shehatae* NJ 23 fermented xylulose at a rate of 0.345 gg cells-1h-1, compared with 0.575 gg cells-1 h-1 for glucose. For all six strains, the addition of glucose to the xylulose medium did not enhance the consumption of xylulose, but increased the cell biomass concentrations. When fermentation was performed under oxygen-limited conditions, less xylulose was consumed by *S. cerevisiae* ATCC 24860 and *C. shehatae* NJ 23, and 50%-65% of the assimilated carbon could not be accounted for in the products determined.  
CC Cytology and Cytochemistry - Plant \*02504  
Comparative Biochemistry, General \*10010  
Biochemistry - Gases \*10012  
Biochemical Methods - General \*10050  
Biochemical Studies - General \*10060  
Biochemical Studies - Carbohydrates \*10068  
Metabolism - General Metabolism; Metabolic Pathways \*13002  
Metabolism - Energy and Respiratory Metabolism \*13003  
Metabolism - Carbohydrates \*13004  
Nutrition - Carbohydrates \*13220  
Microbiological Apparatus, Methods and Media \*32000  
Food and Industrial Microbiology - Biosynthesis, Bioassay and Fermentation \*39007  
Food and Industrial Microbiology - General and Miscellaneous \*39008  
Plant Physiology, Biochemistry and Biophysics - Nutrition \*51504  
Plant Physiology, Biochemistry and Biophysics - Respiration, Fermentation \*51508  
Plant Physiology, Biochemistry and Biophysics - Growth, Differentiation \*51510  
Plant Physiology, Biochemistry and Biophysics - Metabolism \*51519  
Plant Physiology, Biochemistry and Biophysics - Apparatus and Methods \*51524  
BC Ascomycetes 15100  
Fungi Imperfecti or Deuteromycetes \*15500

LH

IT Major Concepts  
Biochemistry and Molecular Biophysics; Bioenergetics (Biochemistry and Molecular Biophysics); Bioprocess Engineering; Cell Biology; Development; Metabolism; Methods and Techniques; Nutrition

IT Chemicals & Biochemicals  
XYLULOSE; ALCOHOL; CARBON

IT Miscellaneous Descriptors  
ALCOHOL PRODUCTION; BIOTECHNOLOGY; CARBON ASSIMILATION; CARBON SOURCE; CELL BIOMASS; MEDIA; METABOLISM; METHODS; SUGAR CONSUMPTION RATES

ORGN Super Taxa  
Ascomycetes: Fungi, Plantae; Fungi - Unspecified: Fungi, Plantae;

Fungi  
Imperfecti or Deuteromycetes: Fungi, Plantae

ORGN Organism Name  
fungus (Fungi - Unspecified); *Candida shehatae* (Fungi Imperfecti or Deuteromycetes); *Pichia stipitis* (Ascomycetes); *Saccharomyces cerevisiae* (Ascomycetes)

ORGN Organism Superterms  
fungi; microorganisms; nonvascular plants; plants

RN 551-84-8Q (XYLULOSE)  
5962-29-8Q (XYLULOSE)  
64-17-5 (ALCOHOL)  
7440-44-0 (CARBON)

L4 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 1993:250914 BIOSIS  
DN PREV199395130089  
TI Cofermentation of glucose and xylose to ethanol by a respiratory-deficient mutant of *Saccharomyces cerevisiae* co-cultivated with a **xylose-fermenting yeast**.  
AU Laplace, Jean M.; Delgenes, Jean P. (1); Moletta, Rene; Navarro, Jean M.  
CS (1) Institut National Recherche Agronomique, Laboratoire Biotechnol.  
Environnement IAA, Boulevard General de Gaulle, 11100 Narbonne France  
SO Journal of Fermentation and Bioengineering, (1993) Vol. 75, No. 3, pp. 207-212.  
ISSN: 0922-338X.  
DT Article  
LA English  
AB As a part of the alcoholic conversion of lignocelluloses, fermentation of a glucose-xylose mixture by a coculture process was investigated in oxygen-limited conditions. In batch mixed cultures of *Saccharomyces cerevisiae* CBS 1200 and *Candida shehatae* ATCC 22984, ethanol was produced only from glucose. During the fermentation by **S. cerevisiae** consuming glucose, the fermentation and growth activities of the **xylose-fermenting yeast** were extremely low, although an optimal condition of oxygen transfer rate in the co-culture was used. The use of a respiratory-deficient mutant of **S. cerevisiae** CBS 1200 allows significant cell growth of *C. shehatae* in a batch culture under a favourable oxygen condition. The growth of *C. shehatae*, however, results in the utilization of glucose, due to the catabolic repression of glucose on the xylose consumption. When the two yeast strains were co-cultivated in a continuous culture, the simultaneous conversion of glucose and xylose was obtained: conversion yields of glucose and xylose were respectively 100% and 27% of a diffusion rate of 0.02 h<sup>-1</sup>. When the mutant of **S. cerevisiae** was co-cultivated with *Pichia stipitis* NRRL Y11545, a rapid **xylose-fermenting yeast**, the co-fermentation of glucose and xylose was enhanced: ethanol was produced with a yield of 0.42 g of ethanol/g of consumed sugars and the respective yields of glucose and xylose conversions were 100% and 69% of the tested dilution rate of 0.02 h<sup>-1</sup>. The advantages of the co-cultivation of a respiratory-deficient mutant of hexose-fermenting and a **xylose-fermenting yeast** are discussed.  
CC Cytology and Cytochemistry - Plant \*02504  
Genetics and Cytogenetics - Plant \*03504  
Comparative Biochemistry, General 10010  
Biochemistry - Gases \*10012  
Biochemical Methods - General 10050  
Biochemical Methods - Carbohydrates 10058  
Biochemical Studies - General \*10060  
Biochemical Studies - Carbohydrates \*10068  
Biophysics - General Biophysical Studies 10502  
Metabolism - General Metabolism; Metabolic Pathways \*13002  
Metabolism - Energy and Respiratory Metabolism \*13003  
Metabolism - Carbohydrates \*13004  
Nutrition - General Studies, Nutritional Status and Methods 13202  
Nutrition - Carbohydrates 13220  
Microbiological Apparatus, Methods and Media 32000  
Food and Industrial Microbiology - Biosynthesis, Bioassay and Fermentation

\*39007  
Plant Physiology, Biochemistry and Biophysics - Nutrition 51504  
Plant Physiology, Biochemistry and Biophysics - Respiration, Fermentation  
\*51508  
Plant Physiology, Biochemistry and Biophysics - Metabolism \*51519  
Plant Physiology, Biochemistry and Biophysics - Chemical Constituents  
51522  
Plant Physiology, Biochemistry and Biophysics - Apparatus and Methods  
51524  
Plant Physiology, Biochemistry and Biophysics - General and Miscellaneous  
\*51526  
BC Ascomycetes 15100  
Fungi Imperfecti or Deuteromycetes \*15500  
IT Major Concepts  
    Biochemistry and Molecular Biophysics; Bioenergetics (Biochemistry and  
    Molecular Biophysics); Bioprocess Engineering; Cell Biology; Genetics;  
    Metabolism; Physiology  
IT Chemicals & Biochemicals  
    GLUCOSE; XYLOSE; ETHANOL; OXYGEN  
IT Industry  
    biotechnology industry  
IT Miscellaneous Descriptors  
    DILUTION RATE; FERMENTATION; GENETICS; METHODS; OXYGEN TRANSFER RATE;  
    RESPIRATION; SUGAR  
ORGN Super Taxa  
    Ascomycetes: Fungi, Plantae; Fungi - Unspecified: Fungi, Plantae;  
Fungi  
    Imperfecti or Deuteromycetes: Fungi, Plantae  
ORGN Organism Name  
    fungus (Fungi - Unspecified); Candida shehatae (Fungi Imperfecti or  
    Deuteromycetes); Saccharomyces cerevisiae (Ascomycetes)  
ORGN Organism Superterms  
    fungi; microorganisms; nonvascular plants; plants  
RN 50-99-7 (GLUCOSE)  
58-86-6Q (XYLOSE)  
25990-60-7Q (XYLOSE)  
64-17-5 (ETHANOL)  
7782-44-7 (OXYGEN)

L9 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2001 ACS  
AN 1989:22273 CAPLUS  
DN 110:22273  
TI Construction of pentose-fermenting strains of *Saccharomyces*  
AU Hollenberg, C. P.  
CS Inst. Mikrobiol., Univ. Duesseldorf, Duesseldorf, D-4000, Fed. Rep. Ger.  
SO Monogr. - Eur. Brew. Conv. (1987), 12, 199-208  
CODEN: MEBCD6; ISSN: 0255-7045  
DT Journal  
LA English  
CC 16-5 (Fermentation and Bioindustrial Chemistry)  
Section cross-reference(s): 3  
AB The classical organism for ethanol prodn., *Saccharomyces cerevisiae*, uses hexoses as a major substrate. The latter constitutes 70% of the prodn. price. Only cheaper substrates can have a large impact on the costs of this process. In this paper possibilities will be addressed to develop yeast strains which can ferment carbohydrates that are not fermentable at present by *S. cerevisiae*. As an example, the possibilities to develop a **xylose-fermenting yeast** strain will be described. Xylose is the monomer of xylan, which constitutes about 10-35% of plant biomass. Expts. towards the introduction of the bacterial xylose isomerase (XI) pathway into *S. cerevisiae* are described. The xylose isomerase gene from *Bacillus subtilis* was isolated and expressed in *S. cerevisiae* under control of the PDC1 promoter. Transformants produced about 2% of the cell protein as the product of the XI gene, but no enzymic activity was detectable. Another approach to introduce the xylose pathway found in some yeasts is discussed.  
ST ethanol fermn xylose *Saccharomyces* gene cloning; *Bacillus* xylose isomerase  
gene cloning yeast  
IT Fermentation  
(ethanol, from xylose by *Saccharomyces cerevisiae*, gene cloning in)  
IT Gene and Genetic element, microbial  
RL: BIOL (Biological study)  
(for xylose isomerase, of *Bacillus subtilis*, cloning and expression in *Saccharomyces cerevisiae* of)  
IT Molecular cloning  
(of xylose isomerase gene, of *Bacillus subtilis*, in *Saccharomyces cerevisiae*)  
IT *Bacillus subtilis*  
(xylose isomerase gene of, cloning and expression of, in *Saccharomyces cerevisiae*)  
IT *Saccharomyces cerevisiae*  
(xylose-fermenting, construction of strains of, for ethanol prodn.)  
IT 58-86-6, Xylose, biological studies  
RL: BIOL (Biological study)  
(ethanol from fermn. of, by *Saccharomyces cerevisiae*, gene cloning in)  
IT 9023-82-9, Xylose isomerase  
RL: BIOL (Biological study)  
(gene for, of *Bacillus subtilis*, cloning and expression in *Saccharomyces cerevisiae* of)  
IT 64-17-5P, Ethanol, biological studies  
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)  
(manuf. of, from xylose by *Saccharomyces cerevisiae*, gene cloning in)

Examiner's ref.

13 ANSWER 1 OF 1 EUROPATFULL COPYRIGHT 2001 WILA

GRANTED PATENT

AN 527758 EUROPATFULL ED 19980119 EW 199802 FS PS  
 TIEN RECOMBINANT YEASTS CONTAINING THE DNA SEQUENCES CODING FOR XYLOSE  
 REDUCTASE AND XYLITOL DEHYDROGENASE ENZYMES.  
 IN HALLBORN, Johan, Vildandsvaegen 2 U: 304, S-222 34 Lund, SE;  
 PENTTILAE, Merja, Vanha Haemeenkylaantie 5-7 A 7, SF-00390 Helsinki,  
 FI;  
 . OJAMO, Heikki, Kirjurinkuja 3 D 25, SF-02600 Espoo, FI;  
 . WALFRIDSSON, Mats, Aellingavaaegen 9 A: 504, S-222 34 Lund, SE;  
 . Airaksinen, Ulla, Lehdochkitie 8 B 26, SF-01300 Vantaa, FI;  
 . KERAeNEN, Sirkka, Rahakamarinkatu 4 B 12, SF-00240 Helsinki, FI;  
 . HAHN-HAEGERDAL, Baerbel, Oestra Martensgatan 5, S-223 61 Lund, SE  
 PA XYROFIN OY, Kyllikinportti 2, 00240 Helsinki, FI  
 PAN 1313873  
 AG Woods, Geoffrey Corlett et al, J.A. KEMP & CO. 14 South Square Gray's  
 Inn, London WC1R 5LX, GB  
 AGN 48721  
 OS EPB1998001 EP 0527758 B1 980107  
 SO Wila-EPS-1998-H02-T1  
 DT Patent  
 LA Anmeldung in Englisch; Veröffentlichung in Englisch  
 DS R AT; R BE; R CH; R DE; R DK; R ES; R FR; R GB; R GR; R IT; R LI; R LU;  
 R NL; R SE  
 PIT EPB1 EUROPÄISCHE PATENTSCHRIFT (Internationale Anmeldung)  
 PI EP 527758 B1 19980107  
 OD 19930224  
 AI EP 1991-906996 19910408  
 PRAI FI 1990-1771 19900406  
 RLI WO 91-FI103 910408 INTAKZ  
 WO 9115588 911017 INTPNR  
 REN Curr Genet, Vol. 18, September 1990, PETER KOETTER et al.: "Isolation  
 and characterization of the *Pichia stipitis* xylitol dehydrogenase gene,  
 XYL2, and construction of a xylose-utilizing *Saccharomyces cerevisiae*  
 transformant", see page 493 - page 500. Appl. Biochemistry and  
 Biotechnology, Vol. 26, No. 2, 1990, VINA W. YANG et al.: "Purification  
 and Properties of Xylitol Dehydrogenase from the Xylose- Fermenting  
 Yeast *Candida shehatae*", see page 197 - page 206, specially the  
 Abstract  
 and the discussion. Journal of Fermentations and Bioengineering, Vol.  
 67, No. 1, 1989, MANFRED RIZZI et al.: "Purification and Properties of  
 the NAD-Xylitol-Dehydrogenase from the Yeast *Pichia stipitis*", see page  
 20 - page 24, see the Abstract. Curr Genet, Vol. 16, 1989, JUTTA  
 HAGEDORN and MICHAEL CIRIACY: "Isolation and characterization of xyl  
 mutants in a xylose-utilizing yeast, *Pichia stipitis*", see page 27 -  
 page 33, see specially page 32, column 2. Process Biochemistry, 1989,  
 BERNARD ALEXANDER PRIOR et al.: "Fermentation of D- xylose by the  
 Yeasts  
 . *Candida shehatae* and *Pichia Stipitis* Prospects and Problems", see page  
 . 21 - page 32, see specially page 24. Enzyme Microb. Technol., Vol. 12,  
 . January 1990, N.W.Y. HO et al.: "Purification, characterization, and  
 . amino: terminal sequence of xylose reductase from *Candida shehatae*",  
 see  
 . page 33 - page 39, see especially pages 35-36, Table 2 page 37, Fig. 5  
 . page 38. Appl. Microbiol. Biotechnol., Vol. 29, 1988, MANFRED RIZZI et  
 . al.: "Xylose fermentation by yeasts", see page 148 - page 154, see  
 . especially discussion page 153, column 2  
 IC ICM C12N015-53

ICS C12N009-04

CM

W1

FA

RLI; AG; REN

DETDEN; CLMEN; CLMDE; CLMFR

PGC

41

CLMN

1

11 ANSWER 2 OF 2 USPATFULL  
AN 85:22459 USPATFULL  
TI Direct fermentation of D-xylose to ethanol by a xylose-fermenting yeast  
mutant  
IN Gong, Cheng-Shung, West Lafayette, IN, United States  
PA Purdue Research Foundation, West Lafayette, IN, United States (U.S.  
corporation)  
PI US 4511656 19850416  
AI US 1982-376731 19820511 (6)  
DCD 20000111  
RLI Continuation-in-part of Ser. No. US 1981-263925, filed on 15 May 1981,  
now patented, Pat. No. US 4368268  
DT Utility  
REP US 1857429 May 1932 435/163.000 Christensen  
US 2481263 Sep 1949 435/153.000 Tsuchiya et al.  
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*Examiner's ref*

L10 ANSWER 2 OF 2 USPATFULL  
AN 1999:15739 USPATFULL  
TI Xylose utilization by recombinant yeasts  
IN Hallborn, Johan, Lund, Sweden  
Penttila, Merja, Helsinki, Finland  
Ojamo, Heikki, Espoo, Finland  
Walfridsson, Mats, Lund, Sweden  
Airaksinen, Ulla, Vantaa, Finland  
Keranen, Sirkka, Helsinki, Finland  
Hahn-Hagerdal, Barbel, Lund, Sweden  
PA Xyrofin Oy, Helsinki, Finland (non-U.S. corporation)  
PI US 5866382 19990202  
AI US 1994-336198 19941103 (8)  
RLI Continuation of Ser. No. US 1992-848694, filed on 9 Mar 1992, now  
abandoned which is a continuation-in-part of Ser. No. US 1990-527775,  
filed on 24 May 1990, now abandoned  
PRAI FI 1990-1771 19900406  
DT Utility  
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DE 4009676 Oct 1991  
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EXNAM Primary Examiner: Chambers, Jasemine C.; Assistant Examiner: Priebe, Scott D.

LREP Birch, Stewart, Kolasch & Birch, LLP

CLMN Number of Claims: 15

ECL Exemplary Claim: 1,9

DRWN 13 Drawing Figure(s); 9 Drawing Page(s)

AB This invention relates to recombinant-DNA-technology. Specifically, this

invention relates to new recombinant yeast strains transformed with xylose reductase and/or xylitol dehydrogenase enzyme genes. A yeast strain transformed with the xylose reductase gene is capable of reducing

xylose to xylitol and consequently of producing xylitol in vivo. If

both of these genes are transformed into a yeast strain, the resultant strain

is capable of producing ethanol on xylose containing medium during fermentation. Further, the said new yeast strains are capable of expressing the said two enzymes. Xylose reductase produced by these strains can be used in an enzymatic process for the production of xylitol in vitro.

PARN This application is a continuation, application Ser. No. 07/848,694 filed on Mar. 9, 1992, now abandoned, which is a continuation-in-part, of application Ser. No. 07/527,775 filed on May 24, 1990.

SUMM FIELD OF THE INVENTION

This invention relates to recombinant-DNA-technology. Specifically this invention relates to new recombinant yeast strains transformed with xylose reductase and/or xylitol dehydrogenase enzyme genes. A yeast strain transformed with the xylose reductase gene is capable of reducing

xylose to xylitol and consequently of producing xylitol in vivo. If

both of these genes are transformed into a yeast strain, the resultant strain

is capable of producing ethanol on xylose containing medium during fermentation.

Further, the said new yeast strains are capable of expressing the said two enzymes. Xylose reductase produced by these strains can be used in an enzymatic process for the production of xylitol in vitro.

ILE 'HOME' ENTERED AT 08:40:33 ON 09 JUL 2001)

FILE 'BIOSIS' ENTERED AT 08:40:48 ON 09 JUL 2001

L1 52 S XYLOSE FERMENTING YEAST  
L2 51 S L1 NOT PY=1999  
L3 48 S L1 NOT PY=1998  
L4 5 S L3 AND S.CEREV рИСИЕ  
L5 0 S XYLOSE FERMENTING S.POMBE  
L6 2 S SCHIZOSACCHARОМYCES POMBE(P)XYLOSE FERMENTING

FILE 'CAPLUS' ENTERED AT 09:15:36 ON 09 JUL 2001

L7 12 S L4  
L8 3 S L6

FILE 'BIOSIS' ENTERED AT 09:17:33 ON 09 JUL 2001

FILE 'CAPLUS' ENTERED AT 09:17:33 ON 09 JUL 2001  
L9 12 S L4

FILE 'USPATFULL' ENTERED AT 09:27:23 ON 09 JUL 2001

L10 2 S L4  
L11 2 S L6

FILE 'EUROPATFULL' ENTERED AT 09:59:14 ON 09 JUL 2001

L12 0 S L4  
L13 1 S L6

FILE 'JAPIO' ENTERED AT 10:07:09 ON 09 JUL 2001

L14 0 S L4 AND L6